

Technical field

This invention belongs to the chemical field, which is related to the fields of pharmaceutical preparations and cosmetic, especially to asiaticoside-liposome and its use for the preparing of pharmaceutical preparations and cosmetic.

Background technology

Centella asiatica(L.)Urban belongs to Umbelliferae. Its herb can be used as an officinal, which has the effects of defervescence, diuretic, detoxicating and anti-swelling etc. As a folk medicine in China, the extract of *Centella asiatica* is used as a remedy for jaundice with damp-heat pathogen, wound and dermal ulcer etc. The existing data indicates that the component of triterpene saponins extracted from *Centella asiatica* can distinctly facilitate the wound healing process, stimulate the growth of the granulation, promote the keratinization of the epidermis, and redound to the generation of new connective tissue, which can also be used as a remedy for burn, the lower limbs' ulcer, wound and adhesion of tendon etc. Moreover, asiaticoside shows significant activity for scar-hyperplasia and keloid, it can prevent skin from erythema induced by ultraviolet irradiation. Therefore it becomes a research hotspot that asiaticoside would be developed into functional cosmetic to prevent and cure cutaneous disease.

Asiaticoside is a triterpene saponin. While practicing use, asiaticoside is found that it can hardly permeate skin because of its big molecular weight(approximate 936), bad liposolubility and water-solubility; asiaticoside is instable in air or solutions and easy to be oxidated and degraded because of the character of its structure, which influence the preparing for stable pharmaceutical preparations and cosmetic

prescription; Moreover, bad liposolubility and water-solubility result in difficulties with the preparation process that asiaticoside can not be mixed with other components of pharmaceutical preparations and cosmetic. These disadvantageous factors restrict the further development and application of asiaticoside in the field of pharmaceutical preparations administered per cutem and cosmetic. Therefore, it is very important to find a kind of suitable drug-carrier which can enhance the chemical stability and skin penetrability of asiaticoside so as to be convenient for the preparation of its pharmaceutical preparations and cosmetic.

One aspect of this invention is to provide a asiaticoside-liposome for skin use, in allusion to the shortcoming lies in asiaticoside's using in pharmaceutical preparations administered per cutem and cosmetic.

Another aspect of this invention is to provide the use of asiaticoside-liposome for preparing pharmaceutical preparations and cosmetic which contain asiaticoside.

Invention content

Asiaticoside-liposome is a kind of opalescent suspension. It is just necessary to mix asiaticoside-liposome with the other components of prescription uniformly when preparing pharmaceutical preparations and cosmetic. The asiaticoside-liposome for skin use is a kind of hydrophilic opalescent suspensions in which asiaticoside is enwrapped in the middle of liposome bilayer membranes. This invention can enhance not only asiaticoside's stability but also its skin penetrability and hydrophilicity, and it is more propitious to prepare pharmaceutical preparations and cosmetic of asiaticoside.

The asiaticoside-liposome for skin use disclosed by this invention is prepared by the following methods and steps:

1. Asiaticoside monomer is isolated from the total saponins of *Centella asiatica* according to conventional methods;

2. The said asiaticoside and lipid components in liposomes prescription are fused by heating or dissolved in organic solvents to make a lipid solution;

3. The said lipid solution is placed into rotary evaporator, then lipid film is afforded at the bottom of the vessel by the rotary thin layer evaporation technique;

4. Lipid dispersing aqueous solution is afforded after the said lipid film had been hydrated by adding aqueous solution under shaking, or afforded by mixing lipid solution mentioned in step 2 with aqueous solution directly under shaking;

5. Asiaticoside-liposome is obtained after the said lipid dispersing aqueous solution has been treated by using the technics of sonication, homogeneous emulsification, microjet and extruding filtration.

Asiaticoside content is 0.1~10% in the asiaticoside-liposome for skin use disclosed by this invention.

In the liposomes prescription of this invention, ceramide is included in the liposomal bilayer structure as an active component.

In addition, at least one kind of the following components should be included in the liposomes: soybean lecithin, yolk lecithin, distearoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, poloxamer, dimyristoyl phosphatidylcholine, tween, span, nonionic surfactant Brij, bile salt, cholesterol.

In the liposomes prescription of this invention, asiaticoside and lipid components of the liposomes account for 0.1~10% and 0.1~40% respectively.

The said organic solvents include dichlormethane, chloroform, aether, ethanol.

The said aqueous solutions include distilled water, deionized water, purified water, phosphate buffer.

A method for the preparation of a liposomal emulsions containing

ceramide is mentioned in CN 98110614.5. Wherein drugs carried by the liposome is provided with stable chemical property, which are difficult to be oxidated, and have the function of skin protection such as damp-keeping, preventing drying and desquamating, etc. These drugs can be easily absorbed by skin. Therefore, the liposomes are perfect as cosmetic additive and drug-carrier for external use. The analogous methods in which liposomes are applied to the preparation of pharmaceutical preparations and cosmetic were disclosed by ZL 96116044.6, CN 96192625.2, CN 93114073.0.

Asiaticoside-liposome of this invention can be applied to the preparation of pharmaceutical preparations and cosmetic. It could be prepared by conventional methods or the methods described in aforementioned patent documents. To form asiaticoside-liposome is useful to enhance the stability, skin penetrability and hydrophilicity of asiaticoside so that it is more convenient and logical to prepare cosmetic or pharmaceutical preparations containing asiaticoside.

Asiaticoside-liposome of this invention is primarily provided with the advantages as undermentioned:

1. To enhance asiaticoside's stability. Drugs are enwrapped in the middle of liposomal bilayer, which can prevent drugs from being destructed by instable factors such as light, oxygen, acid, base and so on, consequently, drugs' stability is enhanced. Liposomes can enhance drug's stability not only in vitro but also in vivo, it can prolong drug's action time in vivo.

2. To enhance asiaticoside's skin penetrability. Liposome are drug carriers composed of lipid bilayer, which has more comparability and compatibility with biological tissue and can enhance drug's skin penetrability. Liposome can not only enhance drug's skin penetrability, but also remain more quantity of drugs between epidermis and dermis, however the dosage entering into hematological system is decreased, so

that general adverse effects can be avoided efficiently. Liposomes can enhance drugs' skin penetrability by the mechanism of hydration, fusion and penetration etc. Furthermore, plentiful ceramides are contained in stratum corneum of human skin. According to similarity-compatibility theory, liposomes containing ceramides in lipid bilayer can further enhance drugs' skin penetrability and absorbability. Asiaticoside-liposome of this invention contain ceramides in the lipid bilayer, they can further enhance asiaticoside's skin penetrability.

3. To be mixed discretionarily with other components in the prescription and make it more simple and convenient to prepare pharmaceutical preparations and cosmetic containing asiaticoside. In prescriptions of most cosmetic, the ground substance is hydrophilic or emulsive, thus components of prescriptions should be hydrophilic or lipophilic. It is difficult to prepare cosmetic containing asiaticoside because of asiaticoside's bad hydrophilicity and lipophilicity. Liposome is a kind of drug carrier with high hydrophilicity, by which asiaticoside is encapsulated and the drug's hydrophilicity is enhanced obviously, the drug can then be mixed discretionarily with other components of the prescription. It is more simple and convenient to prepare pharmaceutical preparations and cosmetic containing asiaticoside.

Detailed examples

Example 1:

30g asiaticoside, 20g soybean lecithin, 30g cholesterol, 40g poloxamer F₆₈, 10g ceramide, 200 ml chloroform, 100ml ethanol and 1000ml phosphate buffer(pH 7.4) were prepared.

Asiaticoside, soybean lecithin, cholesterol, poloxamer F₆₈ and ceramide aforementioned were placed into a 1000ml round bottom flask, dissolved by the mixed solution of chloroform and ethanol, treated with the rotary thin layer evaporation technique in thermostatic waterbath at a

temperature of 25~40°C, and then lipid film was afforded at the bottom of the flask. Then, 800ml phosphate buffer(pH 7.4) was added to the flask, after the lipid film was hydrated under shaking, phosphate buffer(pH 7.4) was added to the mixed solution to 1000ml, then asiaticoside-liposome was afforded after sonification(output4, duty cycle 50%, time 20 mins).

Example 2:

50g asiaticoside, 50g yolk lecithin, 50g cholesterol, 20g ceramide and 1000ml phosphate buffer(pH 7.4) were prepared.

Asiaticoside, yolk lecithin, cholesterol, and ceramide aforementioned were placed into a conical flask and fused by heating or dissolved in organic solvent stated in this invention to make lipid solution, then placed in thermostatic waterbath at temperature 80°C. 800ml phosphate buffer(pH 7.4) was placed in waterbat till its temperature is same as the lipid solution's, then aqueous solution and lipid solution were mixed under shaking and then cooled; phosphate buffer(pH 7.4) was added to the mixed solution to 1000ml, after homogenizing for 6 times with high pressure homogenization technique (higher pressure:60MPa, lower pressure:10MPa), asiaticoside-liposome was afforded.

Example 3:

20g asiaticoside, 20g dipalmitoyl phosphatidylcholine, 30g polydioxivinylcetyether, 40g cholesterol, 40g ceramide, 200ml dichloromethane, 200ml ethanol and 1000ml phosphate buffer(pH 7.4) were prepared.

Asiaticoside, dipalmitoyl phosphatidylcholine, polydioxivinylcetyether, cholesterol and ceramide aforementioned were placed into a 1000ml round bottom flask, dissolved in the mixed solution of dichlormethane and ethanol by heating, treated with the ratary thin layer evaporation technique in thermostatic waterbath at temperature 25~40°C, and then lipid film was afforded at the bottom of the flask. Then, 800 ml phosphate buffer(pH 7.4) was added to the flask,

after the lipid film was hydrated under shaking, phosphate buffer(pH 7.4) was added to the mixed solution to 1000ml. The mixed solution was filtrated extrudedly from poly-(carbonic acid fibrous tunic) and then asiaticoside-liposome was afforded.

Example 4:

Stability experiment

The three groups of asiaticoside-liposome aforementioned and asiaticoside aqueous solution were placed airtight respectively at temperature 40℃, relative humidity 75%. The content of asiaticoside in asiaticoside-liposome and asiaticoside aqueous solution was determined by HPLC after 0, 1, 2, 3 month. The content of asiaticoside in asiaticoside-liposome and asiaticoside aqueous solution was assumed to be 100% at 0 month, the content of asiaticoside at other time was obtained comparing with it at 0 month, then the percentage that the content of drug changed with time was obtained. The result indicated that after placed for three months at temperature 40℃, relative humidity 75%, the content of asiaticoside in asiaticoside-liposome changed a little, but the content of asiaticoside in asiaticoside aqueous solution had decreased. It proved that asiaticoside encapsulated by liposomes could enhance drug's stability obviously.

Table 1 was the comparison of asiaticoside's stability in liposomes and aqueous solution.

Table 1.

The variety percentage of asiaticoside's content (%)				
Time(month)	0	1	2	3
Liposomes	100.00	87.56	75.41	68.02
Aqueous solution	100.00	99.52	98.69	98.12

n=3